

## Original Article

Assessment of antioxidant and antibacterial activity of *Phoenix dactylifera* L. seed extracts: Perspective for the development of new foodsLidia Ait Ouahioune<sup>1</sup>, Fatma Bara<sup>1</sup>, Karim Bariz<sup>2</sup>, Karim Houali<sup>2</sup> , Djamel Djenane<sup>1\*</sup> <sup>1</sup> Laboratory of Food Quality and Food Safety. Mouloud MAMMERI University. Tizi-Ouzou, Algeria<sup>2</sup> Laboratory of Analytical Biochemistry and Biotechnologies. Mouloud MAMMERI University. Tizi-Ouzou, Algeria

## Abstract

**Background:** Conventional food additives were associated with potential harm. Palm seeds are an interesting source of high-added value bioactive compounds, regarding their potential use in food industries. **Aim:** Date seeds extract (DSE) of Algerian *Phoenix dactylifera* L. was studied for its phytochemical, antioxidant, and antibacterial activity and to identify its potential uses in foods. **Material and methods:** A total soluble solid of DSE (°Brix) was determined by refractometry. The total phenolic contents (TPCs) were determined according to the Folin-Ciocalteu method and the total flavonoid and flavonols were also estimated with the colorimetric method. The DPPH method was used to determine the antioxidant activity of DSE. The IC<sub>50</sub> values were also calculated and the antioxidant activity index (AAI) was determined. The agar well diffusion method was employed for the antibacterial activity of the aqueous DSE against various pathogens. **Results:** Moisture, ash, and fat contents (%) determined were 11.06, 1.33, and 7.06, respectively. The physicochemical analysis of the extract showed a pH value of 4.67, titratable acidity value of 0.64 citric acid/100 g, °Brix value = 3%, sugars values of 0.12, 0.47, and 0.35 g/L for reducing sugar, total sugar, and sucrose, respectively. The determination of phenolic compounds was carried out on three groups, namely total phenols = 229.67 mg GAE/g, flavonoids = 201.12 mg QEq/g, and flavonols = 173.03 mg QEq/g, respectively. The DSE demonstrates to be effective as an antioxidant “*in vitro*” and showed a strong antibacterial activity with different inhibition levels, depending on the bacterial strains. **Conclusion:** The current investigations present knowledge about the possible role of palm industry by-products as a novel perspective for the development of new foods. We suggest that date seeds can be exploited in some food applications utilizing their high levels of bioactive molecules.

**Keywords:** *Phoenix dactylifera* L., Degla-Baïda, seeds extract, bioactive molecules, biological activity, *in vitro*, novel perspective.

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## 1 Introduction

The date palm (*Phoenix dactylifera*, Arecaceae: Palmae) is the oldest tree grown in the arid regions and cited in the Holy Quran. The Quran relates that the Virgin Mary was experiencing childbirth pains and then saw a palm tree nearby and held on to it. While she was holding on to the tree, Mary heard a voice telling her “*Shake the trunk of the palm-tree towards you and fresh, ripe dates will drop down onto you*”. Surah Maryam (19:25), and provided huge economic assets, especially in the Middle East and North Africa. According to traditions, all parts of date palms are used in traditional medicine<sup>1</sup>. The *P. dactylifera* fruits play an important role in the economy and social life of people living in arid and semi-arid regions. Algeria is considered to be one of the largest producers of dates in the world where the date palm constitutes a strategic fruit crop containing about one thousand cultivars<sup>2</sup>. However, these genetic resources remain poorly exploited, with the exception of the “*Deglet-Nour*” variety

and to a lesser extent the variety “*Ghars*”, “*Degla-Baïda*” and “*Mech-Degla*”<sup>3</sup>. The date palm fruit is a key agro-product and a vital component of the population’s diet living in the Algerian Sahara. This fruit holds great importance from both nutritional and economic points of view. The industrial processing of this fruit results in the rejection of considerable quantities of waste represented mainly by the seeds. Depending on the variety, date seeds make up about 10 to 15% of the fruit’s weight. In addition, date seeds are used mainly for animal feeds such as camel, cattle, sheep, and poultry<sup>4</sup>. However, the agro chain added-value of this by-product could be considered. Thus, potential applications include oil extraction from the seeds or use them as a dietary-fiber provider in bakery formulations. Ghnimi *et al.*<sup>5</sup> found that date seeds contain about 15% fiber. Another function includes roasting the date seeds and making a caffeine-free drink that can substitute coffee when caffeine is a concern<sup>6</sup>. Chemical and

nutritional constituents of date seeds have been reported in previous studies<sup>7-11</sup>. Date seeds contain a wide range of functional nutritional compounds such as fiber, fat, protein, and vitamins as well as large amounts of phenolic compounds<sup>5,10</sup>. Currently, the interest of public and health professionals has been drawn towards the importance of functional foods in the prevention of illnesses, and as a result, there has been an increase in demand for foods that have the potential of delaying chronic diseases. This is mainly related to the presence of a maximum blend of bioactive compounds, antioxidants, and dietary fiber present in these foods<sup>12,13</sup>. In the past few years, studies on the composition and biological potential of date seeds have increased tremendously. It has been reported that date seeds contain large amounts of polyphenols<sup>13,14</sup>. However, there is a well-known association between the therapeutic activities of date seeds and their chemical constituents. It has been suggested that the date seeds can reduce the risk of cancer and cardiovascular diseases. It was also reported to improve the functionality and integrity of the immune system<sup>15,16</sup>. Conventional food additives were associated with potential harm. Palm seeds are an interesting source of high-added value compounds, which have been stimulating scientific investigation regarding the potential use in food industries. Foods made with natural additives are continuously rising in the food industry to develop new products and re-evaluate the new formulation of processed food. These products containing bioactive molecules can improve health grade by replacing conventional food additives suspected as “harmful”. They are scientific evidence about the actual health advantage of nutraceuticals foods and natural additives. For the future new food generation, date and products play a crucial role. In this context, our study has been carried out to identify and characterize natural bioactive compounds in related *Degla-Baïda* by-products (seeds) and to determine their antioxidant and antibacterial properties.

## 2 Material and Methods

### 2.1 Material

The date seeds were obtained from the *Degla-Baïda* variety recovered as industrial waste during December 2018 from the company Mehiri Dattes (Tolga city, Algeria: Latitude: 34°43'0" N, Longitude: 5°22'0" E, Altitude: 147 m). Four bacterial reference strains (American Type Culture Collection ATCC) which are *Staphylococcus aureus* ATCC 25923 (*S. aureus*), *Pseudomonas aeruginosa* ATCC 27853 (*P. aeruginosa*), *Escherichia coli* ATCC 25922 (*E. coli*), and *Klebsiella pneumoniae* ATCC 4352 (*K. pneumoniae*). All chemicals and reagents employed were of analytical grade.

### 2.2 Preparation of date seed powder (DSP)

Date seeds were washed to remove adherent fruit material. After washing and drying, seeds were grounded into a fine powder. The DSP was stored in opaque glass jars at ambient temperature prior to analysis.

### 2.3 Physicochemical analysis of date seeds powder

The moisture content of DSP was determined by oven drying at 105 °C to constant weight<sup>17</sup>. The fat level was extracted with petroleum ether in the Soxhlet apparatus (Biochem Chemopharma, Cosne-Cours-sur-Loire, France) for 6 h at the boiling point of the solvent (40-60 °C)<sup>18</sup>. To evaluate ash content, about 2 g of DSP was ignited and incinerated in a muffle furnace (Brinder, Germany) at 550 °C for 8 h. All analytical determinations were performed at least in triplicate. The values of different parameters were expressed as the mean  $\pm$  standard deviation ( $X \pm S.D$ ).

### 2.4 Aqueous extraction and preservation of DSE

The extraction was carried out using distilled water by following the protocol of Oomah *et al.*<sup>19</sup> with some modifications. Twenty (20) g of powdered seeds were extracted with 200 mL of distilled water at room temperature for 24 h by a magnetic stirrer (non-heating agitator, Gerhardt; Germany). To obtain an aqueous crude extract, this mixture was filtered and centrifuged at 3000 rpm for 10 min, then the precipitate was removed and the supernatant was lyophilized and stored at  $4 \pm 1^\circ\text{C}$  for further analysis.

### 2.5 Physicochemical analysis of DSE

A total soluble solid measured in degree Brix (°Brix) was determined according to the method of the American Association of Cereal Chemists<sup>20</sup>. The measurements were performed using a refractometer (RHB-90ATC Excelvan; China), after calibration of the device with distilled water. Titratable acidity was determined according to the method NF V 05-101<sup>21</sup>. A quantity of the aqueous extract (25 mL) was taken and then titrated with the sodium hydroxide (NaOH; BiochemChemopharma, Cosne-Cours-sur-Loire, France) solution (0.1 N) in the presence of phenolphthalein (Sigma-Aldrich; Saint-Quentin-Fallavier, France) until a persistent pink color is obtained after 30 seconds. The titratable acidity is expressed in grams of citric acid per 100 g of product. Total sugars and reducing sugars were determined<sup>22</sup>. Non-reducing sugars (sucrose) was calculated as the difference between total sugars and reducing sugars.

### 2.6 Phytochemical analysis of DSE

#### 2.6.1 Measurement of Total Phenolic Contents (TPCs)

The TPCs in DSE were determined according to the method described by Singleton *et al.*<sup>23</sup>. 0.3 mL of the crude extract (1mg/mL) was added to 1.5 mL of Folin-Ciocalteu reagent (Sigma-Aldrich; Taufkirchen, Germany) (1/10), the mixture was incubated for six min and mixed with 1.2 mL of sodium carbonate ( $\text{Na}_2\text{CO}_3$ ; Sigma-Aldrich; Taufkirchen, Germany) (7.5%). The prepared samples were incubated in darkness for two hours and the absorbance was measured at 760 nm. The

result was expressed in mg of gallic acid ( $C_7H_6O_5$ ; BiochemChemopharma, Cosne-Cours-sur-Loire, France) equivalents (GAE) per g of extract.

## 2.6.2 Flavonoid contents

The total flavonoid contents (TFCs) were estimated through the colorimetric method of Quettier-Deleu *et al.*<sup>24</sup> based on the formation of a complex flavonoid-aluminum. About 1 mL of methanolic extract was mixed with 1 mL of 2% hydrated aluminum trichloride ( $AlCl_3 \cdot 6H_2O$ ; Biochem Chemopharma, Cosne-Cours-sur-Loire, France) methanolic solution. After incubation at room temperature for 15 min, the absorbance of the reaction mixture was measured at 430 nm, the TFCs were expressed in mg of quercetin equivalents (QE) per g of extract (mg QE/g).

## 2.6.3 Flavonol contents

The content of flavonols was determined according to Jimoh *et al.*<sup>25</sup> method with minor modifications. The quercetin calibration curve was prepared by mixing 1 mL of various concentrations of ethanolic solutions of quercetin ( $C_{15}H_{10}O_7$ ; Sigma-Aldrich; Saint-Quentin-Fallavier, France) with 1 mL (1 mg/mL) of hydrated aluminum trichloride ( $AlCl_3 \cdot 6H_2O$ ) and 1.5 mL (50 g/L) of sodium acetate ( $C_2H_3NaO_2$ ; Sigma-Aldrich; Saint-Quentin-Fallavier; France). The absorbance at 440 nm was recorded after 15 min. The same procedure was used for 1 mL of DSE (1 mg/mL) instead of quercetin. All determinations were carried out in triplicates. The flavonol content was expressed in mg of quercetin equivalents (QE) per g of extract (mg QE/g Dry matter).

## 2.6.4 Qualitative phytochemical screening

The DSE was subjected to qualitative chemical tests to identify the presence of chemical constituents present using the following methods: (1) alkaloids: Bouchardat's reagent (Iodine-potassium Iodine); (2) saponins: bubble test; (3) terpenoids: sulfuric acid and chloroform (BiochemChemopharma, Cosne-Cours-sur-Loire, France), according to the method of Amana<sup>26</sup>; Yadav & Agarwal<sup>27</sup>; Aziman *et al.*<sup>28</sup>, respectively.

## 2.7 DPPH radical scavenging activity of DSE

The DPPH (1,1-diphenyl-2-picrylhydrazyl;  $C_{18}H_{12}N_5O_6$ ; Sigma-Aldrich; Taufkirchen, Germany) radical scavenging method was used to determine the antioxidant activity of DSE. Radical scavenging activity (RSA) of the extract was measured according to the procedure described by Brand-Williams *et al.*<sup>29</sup>. Briefly, 50  $\mu$ L of the extract was added to 1950  $\mu$ L (60  $\mu$ M) of the DPPH solution. The decrease in absorbance was determined at 515 nm after incubation for 30 min. All the measurements were performed in triplicate. RSA was expressed as the inhibition percentage (%) of free radical by the sample and calculated as follows<sup>30</sup>:

Scavenging effect (% Inhibition)

$$= [(A(\text{control}) - A(\text{sample})/A(\text{control})) \times 100]$$

Where  $A(\text{control})$  is the absorbance value of the control (1950  $\mu$ L DPPH plus 50  $\mu$ L methanol) and  $A(\text{sample})$  is the absorbance value of the sample (1950  $\mu$ L DPPH plus 50  $\mu$ L sample). The  $IC_{50}$  (concentration providing 50% inhibition) values were calculated from the plotted graph of scavenging activity against the concentrations of the samples. To standardize DPPH results, the antioxidant activity index (AAI) proposed by Scherer and Godoy<sup>31</sup> was calculated as follows:

$$AAI = \text{DPPH concentration in reaction mixture } (\mu\text{g/mL}) / IC_{50} (\mu\text{g/mL})$$

The antioxidant activity is considered poor when  $AAI < 0.5$ , moderate when  $AAI$  between 0.5 and 1.0, strong when  $AAI$  between 1.0 and 2.0, and very strong when  $AAI > 2.0$ .

## 2.8 Antibacterial activity

The agar well-diffusion method, according to Djenane *et al.*<sup>32</sup>, was employed for the determination of the antibacterial activity of the aqueous DSE. The inhibitory spectrum of the extract against four reference strains (*S. aureus*, *E. coli*, *K. pneumoniae*, and *P. aeruginosa*) was studied.

The bacterial strain was cultured overnight at 37 °C in Mueller Hinton agar (MHA, Oxoid, Basingstoke, UK). One milliliter of stock culture was standardized through two successive 24 h growth cycles at  $37 \pm 1$  °C in 9 mL of BraineHeart Infusion Broth (BHIB, Oxoid, Basingstoke, UK). After 48 h, 100 mL of the suspension were then inoculated in fresh BHIB and incubated at  $37 \pm 1$  °C for 12 h to obtain a working fresh culture containing about  $6 \log_{10}$  CFU/mL, determined by measuring transmittance at 600 nm (Spectrophotometer: Spectronic 20 Bausch & Lomb). The bacterial strain was maintained frozen at -80 °C in cryovials and was sub-cultured with every antibacterial test.

Mass inoculation was realized by depositing 1 mL of the suspension of  $6 \log_{10}$  CFU/mL at the bottom of the sterile Petri dishes containing 15 mL of MHA. The contents of the Petri dishes were well mixed and allowed to solidify on the bench. After solidification, wells about 7 mm in diameter were made at a distance of 30 mm each using a Pasteur pipette inverted and flamed at its wide end. Each well was filled with 75  $\mu$ L of diluted extracts (100 mg/mL DMSO 15%). The Petri dishes were left at 4 °C for 4 h to allow diffusion of the extract and then were incubated at 37 °C for 18-24 h. The antibacterial activity was expressed as the diameter of inhibition zones in mm produced by the extract against test microorganisms. Negative control (75  $\mu$ L of Dimethyl sulfoxide: DMSO);  $(CH_3)_2SO$  at 15%; Biochem Chemopharma, Cosne-Cours-sur-Loire, France) and the positive (Gentamicin antibiotic; Bioanalyse, India) were used under the same conditions. The experiment was repeated three times.

## 2.9 Statistical analysis

Statistical analysis was performed by Stat-box version 6.2 software using the variance analysis test (ANOVA). This test allowed us to check whether the samples come from the same population or have significant differences. If there is a significant difference, the ANOVA test is followed by the Newman-Keuls complementary test in order to establish the different homogeneous groups. The significance level was 5%.

## 3 Results

For the experimental design, the extract was obtained from Algerian date palm “*Degla-Baïda*”: white date seeds (*Phoenix dactylifera* L.) variety and was studied for its phytochemical, biological activity to identify its potential uses in foods.

**Table 1:** Chemical composition of *Degla-Baïda* (*Phoenix dactylifera* L.) seeds powder

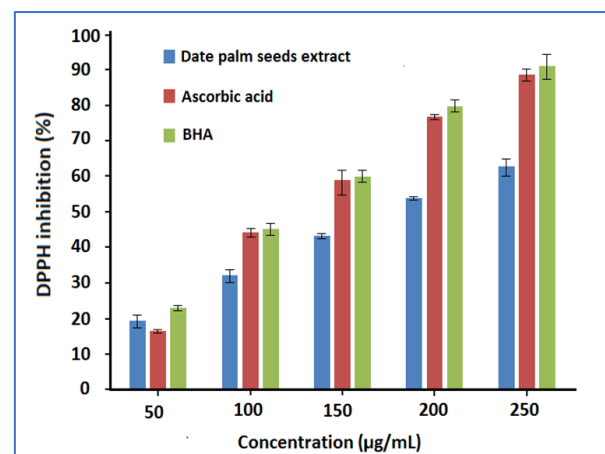
Cultivar	Physicochemical characteristics of date seeds powder (DSP)		
	Ash content (%)	Moisture content (%)	Fat content (%)
<i>Degla-Baïda</i>	1.33 ± 0.28	11.06 ± 0.04	7.056 ± 0.015

Values given are the means of three replicates ± standard deviation

### 3.1 Physicochemical analysis of DSP

The chemical composition of the analyzed DSP is summarized in Table 1. The results of moisture, ash, and fat contents found in the current study are within the range of values indicated earlier in the literature<sup>6,9</sup>. The moisture content (%) of the studied DSP variety is relatively low (11.60 ± 0.04%). Our results agree with those obtained by Habib & Ibrahim<sup>33</sup> who reported values between 8.64 to 12.45% (Emirate cultivar). However, our results are lower than those reported by Al-Farsi et al.<sup>10</sup> regarding *Fard*, *Khasab*, and *Khalas* varieties (Omani cultivar) with values of 18.50, 16.50, and 12.60%, respectively. The average value of fat content in DSP was 7.06 ± 0.02%. This value is similar than to the previous result reported by Hosni et al.<sup>34</sup> who found values of 8.05%. The ash value (1.33 ± 0.28%) was close to those obtained by Saafi-Ben Salah et al.<sup>35</sup> for seven varieties grown in Tunisia (0.97 – 1.17%), and it is also similar to that reported by Khali et al.<sup>3</sup> for the same variety of *Degla-Baïda* (1.10%). In another study on the characteristics of date seeds, Al-Farsi et al.<sup>4</sup> described that date seeds contained 22.5–80% dietary fiber, 3–7% moisture, 2–6% protein, 5–13% fat, and 0.9–1, 8% ash. Hamada et al.<sup>7</sup> reported that the fiber concentration of date seeds varied between 65% and 69%, depending on the variety. Aldhaheri et al.<sup>8</sup> reported a value of 58%, of which 53% were insoluble fibers (cellulose, hemicelluloses, and lignin). On the other hand, Hamada et al.<sup>7</sup>; Herchi et al.<sup>36</sup> found a considerable amount of protein in date seeds (globulin, glutelin, albumin, prolamin). Besides, Herchi et

al.<sup>36</sup> found that carbohydrates were the predominant component in date seed of Kentichi Tunisian variety (83.50%).



**Figure 1:** Antioxidant properties of the *Degla-Baïda* (*Phoenix dactylifera* L.) seed extracts measured by DPPH scavenging method (mean values ± standard deviation)

The observed differences in the chemical composition of date seeds among the varieties grown in the same country or in different regions can probably be attributed to the cultivars, period of harvest, post-harvest treatments, the use of fertilizers, and also climatic factors<sup>33,37</sup>. Moreover, the type of solvent used, the ratio solvent/sample, contact time, and temperature significantly influence the extraction efficacy. In this context, the date seed oil (Kentichi) was examined by Herchi et al.<sup>36</sup>. The authors highlighted that the physicochemical properties of the date palm oil were significantly correlated with temperature, solvents, and extraction protocol.

### 3.2 Physicochemical analysis of DSE

Table 2 shows the physicochemical analysis results of the aqueous DSE. The yield extraction was 4.60 ± 0.03%. This result is different compared to that found in previous studies<sup>38,39</sup>. It is important to underline that the yield extraction of the phenolic extract depends on the method utilized; the selection of solvents, as well as the conditions under which the extraction is carried out (hot or cold). Furthermore, a bad choice could negatively influence the total content of secondary metabolites and therefore decrease the biological activities of obtained extract<sup>4</sup>.

The physicochemical characteristics of the DSE revealed a pH value of 4.67 ± 0.02, total soluble solids contents of 3.00 ± 0.00%, titratable acidity value of 0.64 ± 0.01 g citric acid/100 g date seeds, total sugars content of 47.20%, reducing sugars content of 12.10% and sucrose content of 34.90%.



**Table 2:** Physicochemical characteristics and phytochemical screening of *Degla-Baïda* (*Phoenix dactylifera* L.) seed aqueous extract

Degla-Baïda ( <i>Phoenix dactylifera</i> L.) seed aqueous extract						
Physicochemical characteristics						
Extraction Yield (%)	pH	Titrateable Acidity (citric acid / 100 g)	Brix degree (%)	Total sugar (g/L)	Reducing sugar (g/L)	Sucrose (g/L)
4.6±0.03	4.67±0.02	0.64±0.01	3.00±0.00	0.47±0.01	0.12±0.00	0.35±0.01
Phytochemical screening						
Total phenolic (mg GARq/g)	Flavonoid (g)	Flavonol (g)	Saponins	Alkaloids	Terpenoids	
229.67±0.30	201.12±0.02	173.03±0.12	++	+	+	

++: presence; +: moderate presence

Values given are the means of three replicates ± standard deviation.

This is not consistent with those reported by Khali *et al.*<sup>3</sup> who reported a pH value of  $5.91 \pm 0.19$  and total sugars value of  $7.09 \pm 0.74\%$  for the same cultivars from the same region. Likewise, these results are in contrast with the study developed in Mexico<sup>40</sup>, which reported pH =  $6.98 \pm 0.04$ , total soluble solids =  $5.19 \pm 0.15\%$ , titratable acidity =  $0.05 \pm 0.01\%$  (dw), total sugars =  $5.86 \pm 0.20\%$ , reducing sugars =  $4.40 \pm 0.05\%$  and sucrose =  $1.46 \pm 0.15\%$  from the Madjoul cultivar. Saafi-Ben Salah *et al.*<sup>35</sup> reported 0.91-6.6% for reducing sugars, and 0.61-2.98% for non-reducing sugars (sucrose) for seven varieties grown in Tunisia, respectively. Other authors already reported 10.80 and 7.30% of glucose and fructose, respectively<sup>41</sup>. The chemical constituents contained in date seeds are responsible for the distinct flavors among different cultivars and their incorporation as an ingredient increases the nutritional value of some products<sup>42</sup>. A sensory study was conducted by Fikry *et al.*<sup>43</sup> who demonstrated that the addition of roasted date seed powder in the beverage food showed an improvement in taste, appearance, and overall acceptability by consumers. The date seeds from various cultivars contained a significant amount of important minerals<sup>44</sup>.

Date seed oils are distinguished by their high content in polyunsaturated fatty acids (PUFA): oleic acid (41 to 59%). These fatty acids play an essential role in human nutrition; they are involved in a good source of C18:1 fatty acid and in the prevention of heart diseases<sup>16,45,46</sup>. Besbes *et al.*<sup>47</sup> reported that the oxidative stability of date seed oils was higher than that of most vegetable oils and comparable to that of olive oil and can be stored for a long time without deterioration. Therefore, the idea of application of the date seed oil can be developed as a food supplement in new future generation foods.

### 3.3 Phytochemical determination of DSE

The results of the phytochemical screening, total phenol, flavonoids, and flavonols contents of DSE were determined and the results are presented in Table 2.

Ardekani *et al.*<sup>48</sup>; Bouhlali *et al.*<sup>49</sup> revealed that date seeds phenolic and flavones possess a broad spectrum of biological activity. The qualitative analysis of the three secondary metabolites (saponins, alkaloids, and terpenoids) was also reported by Qadir *et al.*<sup>50</sup> on the variety of *Sukkari* (Saudi Arabian). The results showed the presence of saponins which are plant-derived anti-inflammatory compounds that may lower blood cholesterol and prevent heart disease as well as cancers<sup>51</sup>. A moderate presence of alkaloids and terpenoids has been observed, both compounds may have therapeutic effects. Carotenoids, one of the major subclasses of terpenes, act as biological antioxidants, and protect cells and tissues from the damaging effects of free radicals<sup>52</sup>.

High values were obtained of  $229.67 \pm 0.30$  mg GAEq/g,  $201.12 \pm 0.02$  mg QEq/g, and  $173.03 \pm 0.12$  mg QEq/g for the TPCs, flavonoids, and flavonols respectively. Our data confirm previous results reported by Thouri *et al.*<sup>53</sup> who found in their study on two varieties (*Korkobbi* and *Arechti*) a high content of TPCs and flavonoids in the aqueous extract with regards to different solvents used for extraction: aqueous extract > methanol > aqueous acetone > absolute acetone. Mistrello *et al.*<sup>54</sup> found TPCs and flavonoids contents ranged between 20.58-29.83 mg GAEq/g and 12.71-19.32 mg QEq/g on three varieties (*Zahidi*, *Deglet Nour* and *Khouat Allig*). These results were higher than those reported by Metoui *et al.*<sup>55</sup>, who found on 12 varieties from Tunisia that the *Khadhour* variety contained the highest TPCs (95.32 mg GAEq/g), whereas *Lemsi* had the lowest TPCs (51.30 mg GAEq/g). The polyphenol contents of acetone extract of date seeds were 54.55 and 62 mg GAEq/g at 45 and 60 °C, respectively<sup>56</sup>. These findings are lower than our results ( $229.67 \pm 0.30$  mg GAEq/g). Temperature and solvents had a significant effect on the extraction of total polyphenols and the solubility of polyphenols depends on the category of solvents with varied polarity<sup>57,58</sup>. Date seeds vary widely in their chemical profile owing to factors such as origin and variety, maturation stage, processing, and experimental conditions used for analysis<sup>48,59</sup>. Date seeds have great potential as a supplement for nutraceutical products<sup>4</sup>.

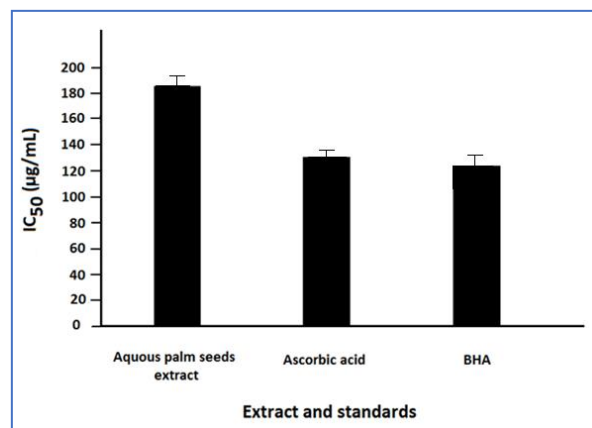
Previous studies have already shown the biological potential of bioactive compounds containing in date seeds.

### 3.4 Antioxidant activity of DES

The antioxidant activity of DSE was estimated using *in vitro* test: the DPPH assay based on the ability of an antioxidant to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. The results showed very highly significant differences ( $p < 0.001$ ) between the percentages of inhibition of DPPH at different concentrations by the aqueous extract, ascorbic acid, and butyl hydroxyanisole (BHA) as given in Figure 1. DSE shows higher antioxidant activity which results in a strong ability to scavenge the DPPH radical ( $62.85 \pm 3.55\%$ ). The amount of antioxidant capable to reduce the concentration of DPPH by 50% ( $IC_{50}$ ) showed very significant differences ( $p < 0.001$ ) between the DSE and the two standards (BHA, ascorbic acid) used. The  $IC_{50}$  values were recorded in the following order: aqueous extract ( $185.56 \mu\text{g/mL}$ ) > acid ascorbic ( $131.42 \mu\text{g/mL}$ ) > BHA ( $125.30 \mu\text{g/mL}$ ) (Figure 2). Scherer and Godoy<sup>31</sup> defined the categories of values corresponding to the antioxidant activity index (AAI) and, according to these categories, *P. dactylifera* seed extracts tested in the present study presented a strong antioxidant activity (AAI = 5.25). In the last few decades, considerable research has been carried out on the antioxidant activity of different varieties of date seeds<sup>38,55,60-63</sup>. Moreover, various studies have already confirmed that different varieties of date seeds constitute an important source of antioxidants and hence their potential use as an ingredient in functional food technology. A study was conducted using flours of date seeds hydrolysates (2.50%) or date seeds (2.5 and 5%) to formulate functional bakery products, such as muffins, in order to determine their potential use as functional ingredients<sup>64</sup>. The same authors found that DPPH radical scavenging activity and hydroxyl radicals were significantly enhanced by both date seeds flour and hydrolysates. In another study on functional pita bread produced with different levels of date seeds powder (5, 15, and 20%), it was noticed that incorporation of higher levels (15 and 20%) of date seeds resulted in a greater amount of phenolics and antioxidant activities as compared with regular and whole wheat bread<sup>65</sup>. Date seeds have also been used as functional ingredients in model meat products<sup>66</sup>. It was reported that antioxidant extracts of date seeds improved the oxidative stability of ground beef by reducing the thiobarbituric acid reactive substances (TBA-RSs: secondary metabolites of lipid oxidation) values of the product as compared to untreated samples. Therefore, these studies strongly suggested that date seeds could be used as potential functional ingredients in the development of new food products. Herchi et al.<sup>36</sup> found that some antioxidants such as ascorbic acid, total phenolic, total flavonoid, chlorophyll, and carotenoids were found in date seed of *Kentichi* (Tunisian variety). El-Sheikh et al.<sup>67</sup> succeeded in incorporating a date seed extract in a beverage and, they concluded that the product obtained presented a better sensory quality compared to coca beverage.

Aiming to characterize the antioxidant status of Tunisian date seed oil, Nehdi et al.<sup>68</sup> found that oil contains liposoluble vitamin type tocopherol 0.60 to 10 mg/100 g. Tocopherols have been recognized as potent natural antioxidants. According to antioxidant mechanisms, it can avoid oxidative damages and reduce the risk of chronic disease. In an investigation, Clemens et al.<sup>69</sup>; El Fouhil et al.<sup>70</sup> reported that date seeds aqueous extract showed a better capacity to protect the human body against genotoxic activity, blood pressure, blood glucose level, and reduced low-density lipoprotein. Bioactive compounds present in beverage date seeds were useful against colon cancer<sup>67</sup>.

Conventional food additives were associated with potential harm. Researchers and professionals in the food industry search for more suitable alternatives to conventional food additives. In this scenario, natural by-products as food additives are receiving particular attention. Palm seeds are an interesting source of high-added value compounds, which have been stimulating the scientific investigation regarding the potential use in the food matrix.

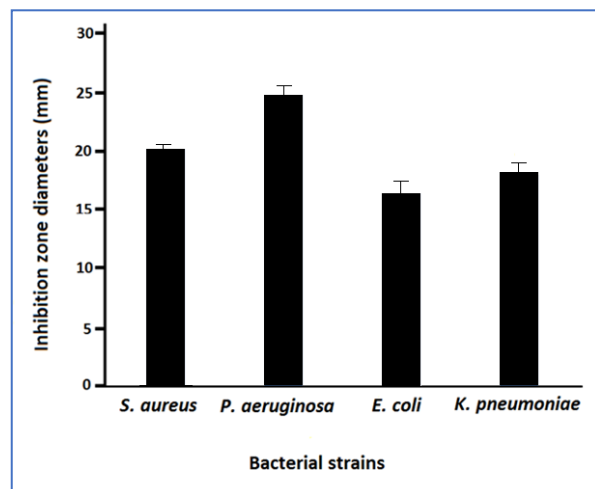


**Figure 2:** The percentage of DPPH• radical scavenging activity of the *Degla-Baïda* (*Phoenix dactylifera* L.) seed extracts expressed as  $IC_{50}$  value ( $\mu\text{g/L}$ ): the concentration required to cause 50% of DPPH inhibition. Different letters indicated a significant difference

### 3.5 Antibacterial activity of DES

Figure 3 shows the antibacterial activity of DSE. The result of the well diffusion method indicated very highly significant differences between the averages of inhibitions zones ( $p < 0.001$ ). The maximum zone of inhibition was observed against *P. aeruginosa* (24.70 mm), followed by *S. aureus* (20 mm), *K. pneumonia* (18 mm), and *E. coli* (16.30 mm). A wide range of antibacterial properties has been reported in different date cultivars when explored *in vitro*. The antibacterial activity of our DSE is very higher than that reported by Metoui et al.<sup>55</sup> on 11 cultivars of date palm seeds from Tunisia who found that the diameter of inhibition of the aqueous extract ranged between 5.30–6.83 mm against *S. aureus*, and 5.10 to 6.70 mm against *E. coli*. Another study on *Halawi*, *Khadrawi*, and *Zahadi*

varieties from Irak reported by Aljazy et al.<sup>71</sup> who found that the aqueous extract of these three varieties showed a weak inhibition against all tested bacteria (6-8 mm).



**Figure 3:** Antibacterial activity of the aqueous extract from *Degla-Baïda* (*Phoenix dactylifera* L.) seed, using paper disc-diffusion method, expressed by diameter (mm) of inhibition zone (including the disc diameter, 6 mm)

Generally, the antibacterial action of date seeds in this report was attributed to its phytochemical compounds that may possibly include phenols, alkaloids, flavonoids, and tannins<sup>72</sup>. Al-Farsi & Lee<sup>73</sup> reported that the phenolic compounds of date seeds such as phenolic acids and flavonoids have been shown to possess many beneficial effects including antibacterial activity, thus it is important to increase the antimicrobial intake in the human diet and one way for achieving this is by enriching food with natural phenolics. Therefore, the use of date seeds as natural antimicrobial agents and their derivatives could be considered an alternative potential solution for many problems as antibiotics-resistant bacteria<sup>74</sup>. Various authors found that date seeds contain antibiotic oxytetracycline whose formation is inducible by *Streptomyces* spp.<sup>75,76</sup>. In the same way and for therapeutic reasons, the Algerian Sahara nomads added date seeds powder as waste to animal feed for goats, camels, and poultry. During the last decades, various plants have been identified for their antibacterial potential in the food matrix. But their availability, characterization, defined mode of action, unknown toxicity level, and cost are the main factors that limit their application at a large scale in the food industry. Nowadays, the scientific approach has explored such sources that are cost-effective and less toxic.

## 4 Conclusion

Exploring agro by-products as sources of high-added value compounds to produce natural food additives can be seen as a promising strategy to replace conventional food additives. From the processing of the Algerian date industry for human consumption, the seeds are the main by-products generated.

Seven phenolic compounds were identified and quantified: TPCs (229.67 mg GAEq/g), flavonoid (201.12 QEg/g), and flavonol (173.03 QEg/g). Date seeds extracts showed an unusual combined antioxidant and antibacterial action, which suggest their great potential as nutraceuticals, particularly as a source of phenolic compounds.

We clearly recognize the limitations of our research article in order to show readers that we are aware of these limitations and to explain how they affect the conclusions that can be drawn from the research. There are three major limitations in this study that could be addressed in future research. First, the study focused only “*in vitro*” evaluation of antioxidant and antimicrobial effects of DSE, second, the exhaustive chemical screening of DSE has not been carried out to identify and characterize volatile and no volatile natural bioactive compounds in related *Degla-Baïda* by-products (seeds), and third, the conclusions of this study justify future research for the application of DSE as biopreservative in food systems to improve their safety and shelf-life by controlling toxigenic and spoilage bacteria.

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## ORCID:

Karim Houali: <https://orcid.org/0000-0002-7317-8657>

Djamel Djenane: <https://orcid.org/0000-0003-4050-4329>

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